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16. ABSTRACT

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TOXICITY OF SYNTHETIC PYRETHROIDS TO FRESHWATER INVERTEBRATES

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Abstract—This report reviews the laboratory toxicity data on freshwater target and nontarget invertebrates exposed to pyrethroids and evaluates the state of the data. Early in their development, pyrethroids were shown to be highly effective insecticides against mosquitoes, blackflies and chironomids. Acute LC50 values for these target animals were often less than 1 μg/L. Acute exposures of nontarget invertebrates showed that some species were as sensitive as the target insects. Longer exposures, up to 28 d, showed that lethal effect concentrations were lowered with increased exposure duration. Unfortunately, the long-exposure tests were limited to a few species and a few pyrethroids, so no general commentary on no effect concentrations is possible. Some species were affected at concentrations near the analytical detection limit for pyrethroids. The data reviewed show that temperature, life cycle stage and exposure duration will be critical factors in developing the no effect concentrations that are important in risk assessments for nontarget invertebrates exposed to these insecticides.

Keywords—Pyrethroids Toxicity Freshwater invertebrates

INTRODUCTION

The ideal pesticide is highly toxic to the target animals but not to nontarget animals, is stable and does not bioaccumulate in the environment. The development of synthetic pyrethroid pesticides appeared to fit these specific requirements [1]. This report evaluates several of these requirements by summarizing and comparing the toxicities of synthetic pyrethroid pesticides to target and nontarget freshwater invertebrates in laboratory studies. The effects of pyrethroids in outdoor systems have been reviewed elsewhere: Mulla et al. [2] included synthetic pyrethroids in their review of the impact of mosquito control agents on nontarget biota in aquatic ecosystems. In 1985, a book on synthetic pyrethroids included an extensive chapter, written by I.R. Hill [3], on the effects on nontarget organisms in terrestrial and aquatic environments. It reviewed laboratory and field data and has special importance because it included data from industry tests that had not been published. More recently, a publication by the Canadian National Resource Council [4] also reviewed the effects of pyrethroids on target and nontarget animals.

Synthetic pyrethroids are pesticides whose chemical structures are based on the chemical structures of the natural pyrethrins. The development of this new form of control agent led to research on their effects in animals. Many of the first reports described target animal bioassays in the laboratory and efficacy tests in ponds or streams. The objective of these studies was to evaluate the pyrethroids' pest control effectiveness. The laboratory data are often presented as LC50 or LC90 values, and for the outdoor studies, as effects per unit of active ingredient per hectare or acre. Later, a group of reports was aimed at evaluating the pyrethroids' effects on nontarget animals. These laboratory studies often included measured concentrations of the agent and reported effects as changes in behavior and death.

TOXICITY TO TARGET ANIMALS

The possible targets for synthetic pyrethroids in freshwater systems are mosquitoes and blackflies. For mosquitoes, laboratory tests were often based

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on the World Health Organization's standard method [5]. Generally, the tests are static and are conducted in small containers with less than 200 ml water. Five concentrations in geometric series are used to expose 20 animals in each of two duplicate containers for 24 h. The endpoint is death and LC50 and LC90 values are calculated using any one of several methods. Fourth-instar larvae are used, although pupae have also been tested. If pupae are used, there is the additional endpoint of adult emergence.

For blackflies, a method described by Muirhead-Thomson [6] was used to evaluate pyrethroid toxicity. This system established wild caught animals in laboratory stream and exposed them for short periods of up to 1 h. The system was designed to simulate the short exposures that occur when a stream is dosed for blackfly control.

Effects in mosquitoes and blackflies

Fourth-instar larvae of two mosquito species were tested with cypermethrin, *cis*-cypermethrin, deltamethrin (decamethrin), permethrin and its cis isomer, and fenvalerate [7]. All the agents were effective at low microgram per liter concentrations. The 24-h LC50s for all four pesticides tested with *Culex* ranged from 0.02 to 7 μg/L. For *Aedes*, the LC50 values ranged from 0.2 to 0.7 μg/L, although permethrin and fenvalerate were not tested. Deltamethrin was the most toxic, while fenvalerate was, in one case, about 350-fold less toxic. In a later report, Mulla et al. [8] evaluated five pyrethroids in six mosquito species. Deltamethrin was again the most toxic, with LC50s ranging from 0.02 to 0.20 μg/L for the six species. Values for *cis*-cypermethrin in 24-h exposures were 0.09 to 0.35 μg/L, so it was slightly less active than deltamethrin. The remaining pyrethroids, permethrin, *cis*-permethrin and fenvalerate, were less active. The LC50s ranged from 0.12 to 3.0 μg/L for *cis*-permethrin and from 0.5 to 3.0 μg/L for permethrin. The least active pyrethroid was fenvalerate, for which the LC50s ranged from 0.9 to 28.0 μg/L. Although there are comparative differences among the five agents, all are highly active against mosquitoes.

Rettich [9] determined the sensitivity of three *Aedes*, two *Culex* and one *Culiseta* species to deltamethrin, permethrin, bioresmethrin and tetramethrin in 24-h static toxicity tests. Deltamethrin was, again, the most toxic to fourth-instar larvae, followed by permethrin, bioresmethrin and tetramethrin for all species, except for a slightly greater sensitivity to bioresmethrin of *A. vexans, Cx. pipiens and Culiseta*. The tetramethrin LC50 range was from 11 to 45 μg/L for *Aedes* and *Culex*. All other LC50s for the various pyrethroid and species combinations were less than 5 μg/L. The LC50s for deltamethrin were usually less than 0.2 μg/L. Pupal sensitivity was equal to larval sensitivity for the four pesticides.

Helson and Surgeoner [10] reported laboratory toxicities for *Culex pipiens, Cx. restuans* and *Aedes stimulans* exposed to cypermethrin. In 24-h tests, the LC50 was 0.073 μg/L for *Cx. restuans* and 0.400 μg/L for *Ae. stimulans* at 20°C. *Cx. pipiens* exposed at 27°C for 24 h had an LC50 of 0.175 μg/L. The 96-h LC50s for larvae and pupae of *Ae. stimulans* exposed at 20°C were 0.229 and 0.158 μg/L, respectively.

The data from these studies show that some mosquito species are acutely sensitive to deltamethrin and cypermethrin at concentrations of less than 1 μg/L. Permethrin, fenvalerate and bioresmethrin are toxic at less than 7 μg/L. Tetramethrin is the least toxic of this group, with LC50 values of greater than 10 μg/L.

Three species of *Simulium* were exposed for periods of 1 h, 30 min and 15 min [6]. The drift of animals from the system during the exposure and survival at 24 h post exposure were used as endpoints. Permethrin at 10 μg/L for 15 min resulted in 86% mortality at the end of 24 h. At 5 μg/L, a 30-min exposure resulted in 89 to 94% mortality. At 1 μg/L, at 1-h exposure produced 60% mortality. Permethrin also acted as an irritant and blackflies drifted from the system. Within 30 min at 1 μg/L, 50% of the larvae had detached. The study also describes a *Simulium* exposure to temephos and reported a 40-fold greater sensitivity to permethrin.

A comparison of *Simulium* larval sensitivities to three pesticides using the 1-h exposure and 24-h survival count was reported by Muirhead-Thomson [11]. Deltamethrin was 4 to 20 times more toxic than chlorphoxim or temephos in these tests. Mortality was greater than 95% when the larvae were exposed to 0.5 μg/L for 1 h. The LC50 for a 1-h exposure to deltamethrin was less than 0.1 μg/L.

Effects on chironomids

Chironomids can be either a target or a nontarget species, depending on their numbers and location. The effect of pyrethroids on chironomids was documented by Ali [12] and Ali and Mulla [13,14]. These studies were initiated to control nuisance emergences of chironomids in residential-recreational lakes and drainage channels in southern California and Florida because the use of the or-

ganophosphate insecticide chlorpyrifos had produced resistant populations. In the first two studies [13,14], field-collected animals were exposed for 24 h in a test system similiar to the mosquito bioassay [5]. Each test lasted for 24 h and exposures were in paper cups containing 100 ml water and 5 g sterilized sand. Both LC50 and LC90 values were calculated, but only the LC50 values are reported here for comparison with other LC50 values.

Four pyrethroids, deltamethrin, *cis*-cypermethrin, *cis*-permethrin and fenvalerate, were tested against three species [13]. In the 12 tests, the LC50s ranged from 0.07 to 1.1 μg/L for deltamethrin, from 0.1 to 1.5 μg/L for *cis*-cypermethrin, from 1 to 24 μg/L for *cis*-permethrin and from 4.2 to 18 μg/L for fenvalerate. The second study evaluated control agents for chironomids in drainage channels. Four pyrethroids were tested against four species that were resistant to organophosphate insecticides. Deltamethrin and *cis*-cypermethrin LC50s ranged from 0.06 to 4.6 μg/L; for *cis*-permethrin, from 4.5 to 19 μg/L; and for fenvalerate, the least toxic, from 11 to 80 μg/L. A ratio of the most toxic organophosphate to the most toxic synthetic pyrethroid for each species showed a range of 19 to 900 times greater sensitivity to the pyrethroids. A similiar experiment with chironomids from another channel produced LC50 values of 0.15 and 1.4 μg/L for deltamethrin against *Cricotopus* spp. and *Dicrotendipes californicus*, respectively. The ratio of the most active organophosphate to the most active pyrethroid for each species showed 23 and 5 times greater activity for the pyrethroid. The last article in this series was an evaluation of pyrethroids for use in Florida. Ali [12] reported the results of tests with six pyrethroids against five species of chironomids. All the pyrethroids were toxic at low concentrations. The LC50s ranged from 6.5 μg/L for *cis*-permethrin to 0.12 μg/L for a numbered product, FMC 52703. Twenty-one tests were done and in 15, or 71%, the LC50 was less than 1 μg/L.

Comparisons of data

The acute sensitivities of target animals to the most commonly tested pyrethroids are summarized in Figures 1 and 2. For mosquitoes, deltamethrin is generally more toxic than cypermethrin, permethrin and fenvalerate. Bioresmethrin and tetramethrin are the least toxic in this group. In Figure 1, 55 values are represented, and 22, or 40%, are greater than 1 μg/L. Bioresmethrin and tetrame-

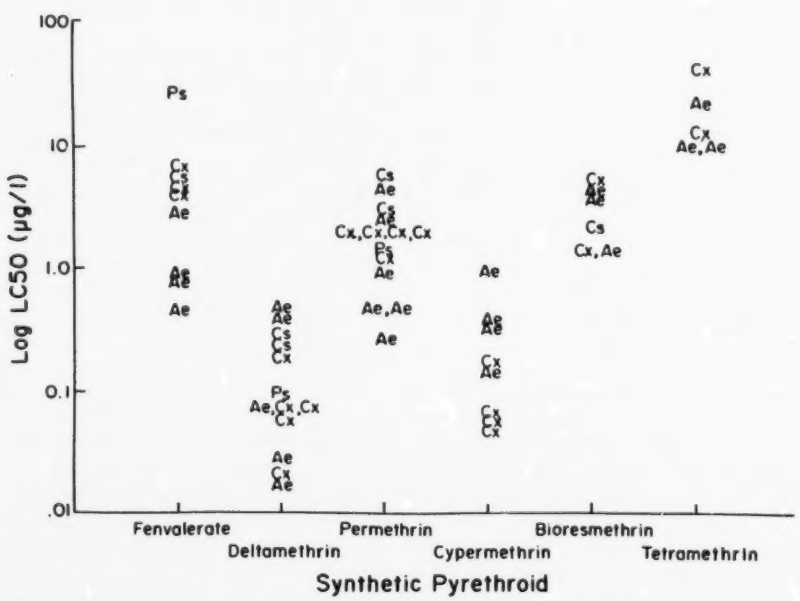

Fig. 1. Summary of acute toxicity values for five pyrethroids in mosquitoes (all for 24-h static exposures). Ae, *Aedes*; Cx, *Culex*; Cs, *Culiseta*; Ps, *Psorphora*. Data from refs. 7–10, 18 and 27.

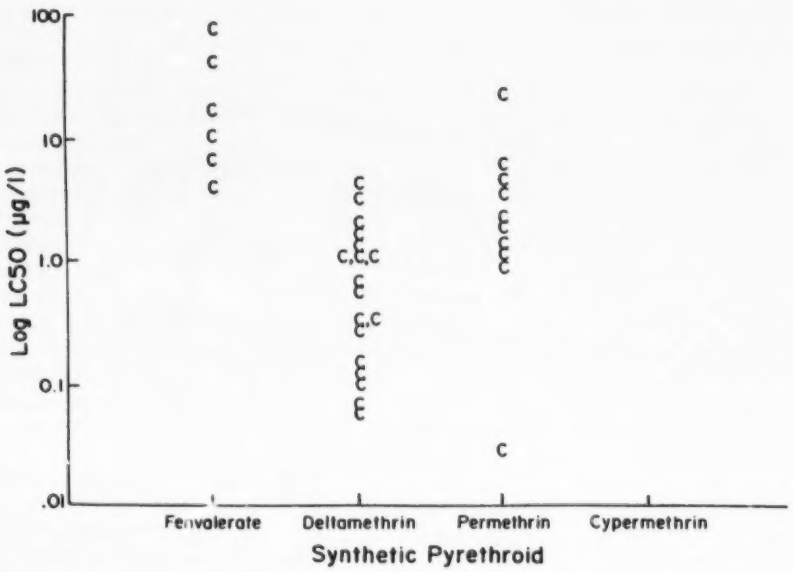

Fig. 2. Summary of acute toxicity values for three pyrethroids in chironomids (all for 24-h static exposures). Data from refs. 12–14.

thrin account for 11 of the 22 values that exceed 1 µg/L. The data for chironomids are shown in Figure 2. Eight genera were tested and all the acute values are combined on the graph for each pyrethroid. Several points are evident. First, there is a wide range of sensitivities. For fenvalerate, the LC50s range from 4.2 to 80 µg/L and for permethrin, the range is from 0.03 to 24 µg/L. Second, there are fewer data for the chironomids than for mosquitoes. Third, deltamethrin is the most toxic pyrethroid tested, based on its lower range of lethal concentrations.

TOXICITY TO NONTARGET INVERTEBRATES

The desire to control aquatic pests resulted in mosquito, blackfly and chironomid tests, but no similiar desire has driven nontarget species tests. While the registration of pyrethroids requires a battery of tests with nontarget animals [15], the required or auxiliary data often are not published. The result is that the number of peer-reviewed reports published on nontarget animal sensitivity to pyrethroids is small. The data are limited to acute and a few longer-duration exposures with a few species, and there is little consistency in exposure procedures. Although the information is slight, the studies do provide an impression of nontarget animal sensitivity.

The effect of deltamethrin on *Tubifex* was reported by Jordan [16], whose study examined embryo development and individuals living inside and outside cocoons. Concentrations greater than 25 mg/L caused a rapid death of embryos and of free-swimming animals. At 250 µg/L, 50% of the animals outside cocoons were killed in 20 d. For *Tubifex* living inside cocoons, the same effect occurred at 25 µg/L. Normal development occurred at 2.5 µg/L, but higher concentrations inhibited development. Some mortality in free-living animals was seen at 0.025 to 0.25 µg/L.

Day and Kaushik [17] conducted a three-part study that evaluated the effects of fenvalerate on survival, filtration and assimilation in three zooplankton species, *Daphnia galeata mendotae, Ceriodaphnia lacustris* and the copepod *Diaptomus oregonensis*. The objective was to study the effects of low concentrations on filtration and assimilation. Effects on filtration were measured by exposing animals to fenvalerate and then allowing them to feed on radiolabeled algae for a short time. The animals were killed after the feeding and the radioactivity was measured. Assimilation was measured

using a similar procedure but after the ingestion of radioactive algae the animals were allowed to feed on nonradioactive algae to clear their guts before radioactivity analysis. Prior to the filtration and assimilation experiments, 24-h and 48-h EC50 values were determined and reported for *Daphnia magna*, as a reference. Acute toxicity increased from 24 to 48 h for all four species. The ratio of 24-h to 48-h EC50s ranged from 1.7 for *D. galeata mendotae* to 5.2 for *C. lacustris*. At 48 h, *D. magna* was the least sensitive, with an EC50 for adults of 2.5 μg/L, as compared with 0.29, 0.21 and 0.12 μg/L for *D. galeata mendotae, C. lacustris* and *D. oregonensis*, respectively. Two 48-h exposures starting with young less than 48 h old showed that these animals were 1.8- to 3.4-fold more sensitive than adults. Exposure to sublethal concentrations of fenvalerate reduced the filtration rates for the two cladocerans and the copepod. However, assimilation rates were not as sensitive an indicator and were affected only at concentrations that were acutely lethal.

A three-article series described the effects of cypermethrin in laboratory and pond exposures. One of the reports [18] described tests with 10 species of invertebrates and 5 species of fish. In these tests, the concentration of cypermethrin was measured and two endpoints, reduced motility and death, were used. The 24-h EC50s ranged from 0.02 μg/L for an isopod (*Asellus*) and water mite (*Piona*) to 2.0 μg/L for *D. magna*. For 7 of the 10 invertebrate species, the 24-h EC50s were less than 0.1 μg/L. Seven of the 10 also had 24-h LC50s of between 0.05 and 2.0 μg/L. For the remaining animals, a beetle (Coleoptera, *Gyrinus*), a fly (Diptera, *Chironomus*) and a bug (Hemiptera, *Corixa*), the LC50s were less than 5 μg/L. The fish exposures lasted 96 h and the LC50 range was narrow, from 0.4 to about 2.2 μg/L. A comparison of invertebrate 24-h and fish 96-h LC50s supports the view that some important fish food invertebrates are more sensitive than the most sensitive fish.

The blackfly rapid-flow short-exposure system [6] was used to test nontarget animals [19]. A 5-μg/L 1-h exposure killed all *Gammarus, Brachycentrus* and *Baetis* and 37% of the *Hydropsyche*. At 1 μg/L for 1 h, 93, 64 and 91% of the *Gammarus, Brachycentrus* and *Baetis*, respectively, were killed, but no *Hydropsyche* were affected. Tests with *Simulium*, a target genus, resulted in 60% mortality after 1 h at 1 μg/L.

Jolly et al. [20] reported that crayfish were more susceptible than fish or amphibians in tests with permethrin. The 96-h LC50 values were 0.39 μg/L for 8- to 12-mm animals and 0.62 μg/L for 20- to 30-mm crayfish. These values were about one-half to one-third the LC50 of the most sensitive fish tested in the experiments

Most of the published data for synthetic pyrethroids describe acute laboratory exposures. Two reports on longer laboratory exposures [21,22] showed that increasing the duration beyond 4 d continues to decrease the toxic effect concentration. Also, low-concentration long-exposure tests allow for expression of a sequence of changes in behavior that lead to immobilization and, in some cases, death. Behavior changes or death were seen with fenvalerate and permethrin [21] at the lowest concentrations tested (0.022 and 0.030 μg/L, respectively). Each pyrethroid was unique in its effect on the behavior of a species. Fenvalerate was rapidly toxic and caused behavior changes faster than did permethrin. At similar concentrations, the intoxication sequence was similar, but once the animals were immobilized fenvalerate was more lethal than permethrin. Permethrin-exposed stoneflies (*Pteronarcys*) were immobilized for up to 21 d and, when transferred to pyrethroid-free water, some recovered.

Flucythrinate's effects on the swimming of *Pteronarcys* were seen as quickly as 2 h after exposure to 0.033 μg/L [22]. After 24 h at 0.007 μg/L, 55% had lost coordinated movement. By the seventh day of exposure, enough stoneflies had died to allow a calculation of an LC50 (0.071 μg/L) and death continued to occur in the immobilized animals until a minimum LC50 of 0.015 μg/L was reached after 18 d. In a similar experiment with *Brachycentrus*, immobilization occurred after 24 h at 0.016 μg/L, but the first LC50 could not be calculated until 12 d (0.069 μg/L). At the end of the exposure at 28 d, the LC50 was 0.020 μg/L. For the longer exposures to fenvalerate, permethrin and flucythrinate, sensitive animals were affected at concentrations near the analytical limit.

Nontarget invertebrate acute data are summarized in Figure 3. Since many species were tested only once, the data are grouped into macroinvertebrate and zooplankton categories. The macroinvertebrate data are 24-h and 96-h acute values and the zooplankton data are 24-h LC50 or EC50 values. For cypermethrin, all values are for 24-h exposures. This summary shows that there are many fewer data than for either mosquitoes or chironomids (Figs. 1 and 2). Only 17 acute values are shown, whereas 55 mosquito and 34 chironomid acute values were found. The few data points make comparative evaluation difficult, but it is

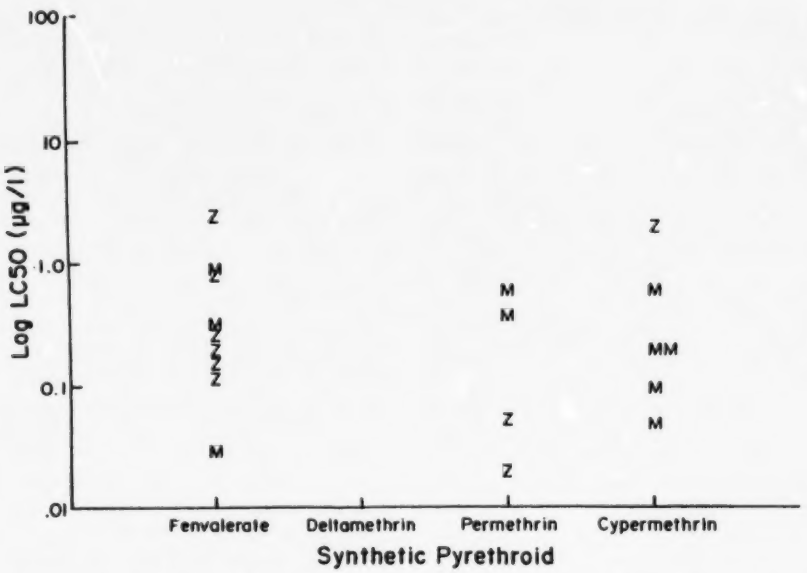

Fig. 3. Summary of acute toxicity values for three pyrethroids in nontarget invertebrates. M, macroinvertebrates; Z, zooplankton. Data from refs. 17, 18, and 20–22.

apparent that certain nontarget animal groups are at least as sensitive as the target animals.

The data from these reports also show that young animals may be more sensitive than adults. Day and Kaushik [17] demonstrated a 1.8- to 3-fold greater sensitivity in young Cladocera, and Anderson [21] and Jolly et al. [20] reported similiar sensitivity ratios between young and old animals for *Gammarus* and crayfish (*Procambarus clarkii*), respectively.

Other studies

Death or immobilization is the major endpoint in almost all of the published studies, but other effects have been reported. Stratton and Corke [23] reported permethrin 48-h LC50 acute values for juvenile and adult *D. magna* of 0.2 to 0.6 µg/L. They also reported that permethrin resulted in the adhesion of particles to the swimming antennae of the *Daphnia*. The degree of adhesion was affected by the particle type and the concentration of permethrin. Algae adhered more readily than bacteria or silica. The cause of the adhesion was not clear, although it appeared that permethrin had reacted with the *Daphnia* setae so that they became sticky. Experiments with other pesticides showed that a similiar adhesion occurs and the authors suggest that this may be a general response of the animal when exposed to the chemicals at sublethal concentrations. Adhesion was also reported by Day and Kaushik [17] in *Daphnia galeata mendotae*, but none was noted in *Ceriodaphnia lacustris* or *Diaptomus oregonensis*. It appears that adhesion is not universal in sublethal exposures and its extent and causes are not settled.

Information on the effects of pyrethroid on embryos is limited. Two reports show that embryos may be more resistant than juveniles or adults. For *Tubifex* exposed to deltamethrin, Jordan [16] reported that the blastula stage was more resistant than fully developed individuals. Anderson and Shubat [24] showed that about 50% of the embryos of the chironomid *Paratanytarsus* did not hatch when exposed to 3.1 µg/L flucythrinate throughout their development. Although this value may appear to be low, lethal concentrations for fully developed animals of several species of invertebrates are 10- to 100-fold lower [22].

The accumulation of pyrethroids by invertebrates in laboratory tests is typically less than 1,000 times the water concentration. Accumulation is affected by factors that affect the actual water concentration such as sorption to sediment, suspended particles and plants, and by degradation of

the molecule by microbes or light. Muir et al. [25] showed that the composition of the sediment could affect uptake by chironomids. If the sediment was sand, the accumulations were 5 to 15 times higher than if the sediment was clay or silt. In addition, some of the pyrethroids were more readily accumulated. The bioconcentration factor (BCF) ranged from 135 for *trans*-permethrin to 316 for deltamethrin. Depuration from these 24-h or 48-h exposures was rapid. The average half-life for residence in the body for the four compounds was 27 h. Anderson [21] reported that snails (*Helisoma*) exposed for 28 d to fenvalerate were unaffected, and the BCF ranged from 356 to 1,200 depending on the exposure concentration. For the stonefly *Pteronarcys* exposed to permethrin, the BCF averaged 183 for a 21-d exposure. Spehar et al. [26] reported no effects but a mean BCF of 800 for *Helisoma* exposed to permethrin.

There is evidence that physical factors can affect toxicity. It appears that exposure at lower temperatures results in greater toxicity. Cutkomp and Subramanyam [27] exposed the mosquito *Aedes aegypti* at two temperatures to six pyrethroids and found that the toxicities were 1.3- to 3.6-fold greater at 20 than at 30°C. Helson and Surgeoner [10] compared the sensitivities of *Culex pipiens* to cypermethrin at 14 and 27°C and found a threefold increase in toxicity at the lower temperature. There are no comparative data for nontarget aquatic invertebrates.

DISCUSSION

The ideal pesticide should have high target animal toxicity, low toxicity to nontarget animals, stability and low accumulation in the environment. This review has shown that, in the laboratory, pyrethroids are highly toxic to specific aquatic nontarget invertebrates. The acute data show that some invertebrates, especially Crustacea and Insecta, are equal to or more sensitive than the potential target insects. The few tests that lasted longer than 4 d show that constant exposure to concentrations of less than 0.5 μg/L can affect behavior and may also be lethal to some species. Some multiple-week exposure data show effects at low nanogram per liter concentrations.

Pyrethroids can enter water either by direct application, or by drift or runoff from use on land. Clearly, if pyrethroids were directly applied to control aquatic pests, the acute data would forecast reductions in some nontarget invertebrate populations. Indeed, such a prediction is supported in reviews [2–4] of the pyrethroids' effects in natural systems. The same confidence cannot be assigned to predictions of effects associated with drift or runoff from land applications. Although it is well known that pyrethroids adsorb strongly to organic material [3], drift or runoff may result in low-concentration exposures of variable duration. Laboratory data for this style of exposure are not available.

What additional research is needed to aid in the assessment of pyrethroids' effects on nontarget freshwater invertebrates? Some pyrethroids are acutely toxic at low concentrations, but these tests have been conducted with only a relatively few species under, with some exceptions, constant exposure conditions of 24 h or longer. To better correlate laboratory exposures with realistic field conditions, additional laboratory tests should be conducted with a diverse assortment of nontarget species and emphasis on the effects of low concentrations and short-term, variable or long-term, extended exposures. This primary screening should be followed by detailed examinations of the effects of biological factors such as life cycle stage and physical factors such as temperature on the toxicity of the pyrethroids in sensitive species.

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